Microdevices to locally electroporatemouse embryos: from the proof-of-concept to the prototype

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During embryonic development interactions between cell groups enable the growth, migration, and specification of the various tissues. The ability to follow and modify cell behavior with accurate spatiotemporal resolution thus appears as a prerequisite to study morphogenesis. Electroporation, the delivery of exogenous molecules into targeted cell populations through electric permeation of the plasma membrane, has for instance been harnessed to manipulate gene expression locally. However, current strategies relying on sharp metallic electrodes suffer from insufficient reproducibility and mediocre survival when applied to early post-implantation mouse embryos. Indeed, harmful species such as gases and protons are produced by electrolysis, which often resultsin the death of thesmall – between 150 and 500 μm long – and delicate organisms.

We thus introducedan "electrodeless" approach in which the electric field is generated by remote gold pads and channeled towards the cells to be transfected by dielectric tunnels. Finite element model simulations relying on a simple electrical model of the mouse embryo indicated that the present strategy performedeven better than the existing alternatives. A microsystem including SU-8 photoresist walls and a Parafilm coverwas next fabricated on a glass wafer. Its performances were tested by targeting the distal visceral endoderm (DVE): penetration of a fluorescent dextran enabled to assert membrane permeation, expression of plasmid coding for a fluorescent protein reported on transfection success, and cell death was evaluated thanks to propidium iodide staining. The size of the dielectric guide aperture as well as the voltage pulse sequence could be optimized in order to efficiently and reproducibly restrict transfection to less than four cells, without compromising cell behavior and embryo survival (Mazari, *et al.*, (2014) *Development*, **141**, 2349).

This SU-8/Parafilmdevice enabled us to study tissue rearrangementin live imaging microscopy, which enforcedthe biological relevance of our technological developments. However, the chip was single-use and had to be fabricated in a clean-room within 48 h before electroporation. To make more accessible the localized introduction of cell markers in developing organisms, we are now transformingthe previous demonstrator in a reusable prototype complying with industrial micromachining techniques and user-inspired ergonomic choices. Design changes are assisted by numerical simulations and are validated with the above set of fluorescent markers, in an integrated engineering approach.